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***SDHC* pheochromocytoma and paraganglioma: a UK-wide case series**

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3 **1 SDHC pheochromocytoma and paraganglioma: a UK-wide case series**

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5 **2 Short title:** *SDHC* variants: a UK-wide case series

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49 70 **Conflicts of Interest Statement**

50  
51 71 No conflicts of interest to declare.

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54 72

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#### 77 **Data availability statement**

78 The data that support the findings of this study are available on request from the corresponding  
79 author. The data are not publicly available due to privacy or ethical restrictions.

#### 81 **Ethics statement**

82 This study was approved by the Guy's and St Thomas' Trust Clinical Audit Team (Service  
83 Evaluation 9649). The study conforms to the Declaration of Helsinki.

#### 85 **Summary**

86 **Objective:** Pheochromocytomas (PCC) and paragangliomas (PPGL) are rare, but strongly  
87 heritable tumours. Variants in succinate dehydrogenase (SDH) subunits are identified in  
88 approximately 25% of cases. However, clinical and genetic information of patients with *SDHC*  
89 variants are under-reported.

90 **Design:** This retrospective case series collated data from 18 UK Genetics and Endocrinology  
91 departments.

92 **Patients:** Both asymptomatic and disease-affected patients with confirmed *SDHC* germline  
93 variants are included.

94 **Measurements:** Clinical data including tumour type and location, surveillance outcomes and  
95 interventions, *SDHC* genetic variant assessment, interpretation, and tumour risk calculation.

96 **Results:** We report 91 *SDHC* cases, 46 probands, and 45 non-probands. Fifty-one cases were  
97 disease-affected. Median age at genetic diagnosis was 43 years (range:11-79). Twenty-four  
98 *SDHC* germline variants were identified including six novel variants. Head and neck



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3 99 paraganglioma (HNPGL, n=30, 65.2%), extra-adrenal paraganglioma (EAPGL, n=13, 28.2%)  
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5 100 and PCC (n=3, 6.5%) were present. One case had multiple PPGLs. Malignant disease was  
6  
7 101 reported in 19.6% (9/46). Eight cases had non-PPGL *SDHC*-associated tumours, six  
8  
9 102 gastrointestinal stromal tumours (GIST) and two renal cell cancers (RCC). Cumulative tumour  
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11 103 risk (95% CI) at age 60 years was 0.94 (CI:0.79-0.99) in probands, and 0.16 (CI:0-0.31) in non-  
12  
13 104 probands respectively.  
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17 105 **Conclusions:** This study describes the largest cohort of 91 *SDHC* patients worldwide. We  
18  
19 106 confirm disease-affected *SDHC* variant cases develop isolated HNPGL disease in nearly 2/3 of  
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21 107 patients, EAPGL and PCC in 1/3, with an increased risk of GIST and RCC. 1/5 developed  
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23 108 malignant disease, requiring comprehensive lifelong tumour screening and surveillance.  
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## 124 ***SDHC* phaeochromocytoma and paraganglioma: a UK-wide case series**

125

### 126 **Introduction**

127 Phaeochromocytoma and paragangliomas (PPGL) are rare, but strongly heritable  
128 neuroendocrine tumours that are associated with a number of hereditary syndromes. European  
129 studies suggest the incidence is approximately 0.6 cases per 100,000 person years.<sup>1</sup> The WHO  
130 guidelines use anatomical location to classify PPGL tumours into adrenal-gland  
131 phaeochromocytoma (PCC), accounting for 80% of PPGL disease and extra-adrenal  
132 paraganglioma (EAPGL) accounting for 20%.<sup>2,3</sup> EAPGL are subdivided into sympathetic  
133 paraganglioma (PGL), associated with catecholamine secretion, and parasympathetic derived  
134 head and neck PGL (HNPPGL) that rarely exhibit catecholamine secretion.

135

136 Over 40% of PPGL patients who undergo genetic testing are found to have a germline  
137 heterozygous pathogenic variant in a PPGL susceptibility gene.<sup>4</sup> Germline loss-of-function  
138 variants affecting genes encoding subunits of succinate dehydrogenase (SDH), which also  
139 functions as Complex II in the electron transport chain, are identified in 25% of PPGL cases,  
140 resulting in different autosomal dominant syndromes.<sup>5</sup> Loss of *SDHB* expression is detectable  
141 when any component of the SDH complex is inactivated, but no specific, validated *SDHC*-  
142 specific immunohistochemistry (IHC) exists.<sup>6</sup>

143

144 In the UK, patients with PPGLs are recommended to be referred for genetic counselling.  
145 Genomic testing is available if they fulfil National Genomic Test Directory criteria.<sup>7,8</sup> Eligible  
146 patients are currently offered an 11 PPGL panel genomic test (*SDHA*, *SDHB*, *SDHC*, *SDHD*,  
147 *SDHAF2*, *FH*, *MAX*, *MEN1*, *RET*, *TMEM127* and *VHL*). Variants of unknown significance  
148 (VUS) are reported in approximately 10% of PPGL diagnostic tests. If a pathogenic variant is

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3 149 identified, familial cascade genetic screening testing can be offered. Surveillance of  
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5 150 asymptomatic relatives includes Magnetic Resonance Imaging (MRI) and biochemical testing  
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8 151 for catecholamine secretion, to aid early detection of tumours.<sup>7</sup> The optimal surveillance  
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10 152 programme is debated and numerous protocols exist.<sup>9,10</sup>

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14 154 *SDHx*-associated disease characteristics vary according to the pathogenic SDH-subunit. *SDHB*  
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16 155 variants are most prevalent, accounting for 10% of PPGL cases, *SDHD* variants 5%-9%, *SDHC*  
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18 156 1-2% and *SDHA* <1%.<sup>9</sup> *SDHB* variants are most common in patients with PCC or EAPGL,  
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20 157 while *SDHD* variants frequently present with multifocal HNPGL.<sup>11</sup> The risk of aggressive,  
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22 158 malignant disease is highest in patients *SDHB* pathogenic variants.<sup>12</sup> *SDHC* PPGL-associated  
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24 159 pathogenic variants are less frequent than *SDHD* or *SDHB*, predominantly presenting with  
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26 160 HNPGL disease at a younger age.<sup>11,13,14</sup> Less is known about *SDHC*-associated disease, with  
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28 161 respect to clinical behaviour, disease penetrance, non-PGL associated pathology and optimal  
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30 162 surveillance.

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37 164 There are three objectives of this nationwide series: First to describe the demographic, clinical  
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39 165 and treatment data for both proband and non-proband individuals. Secondly to report genetic  
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41 166 variants implicated in *SDHC*-associated PPGL disease and use structural analyses to evaluate  
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43 167 pathogenicity in novel variants. Thirdly, to evaluate current surveillance practice and make  
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45 168 recommendations for future care, based on the data presented.

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## 50 51 170 **Materials and methods**

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53 171 This retrospective study presents genetic and clinical information of germline *SDHC* variants  
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55 172 (OMIM 602413, reference sequence NM\_003001.3), diagnosed before March 2020, from 18  
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57 173 UK centres. An invitation to participate was sent to 23 UK centres in April 2019 (see  
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3 174 Appendix). Follow-up ended March 31st 2020, or if lost to follow up, date of last hospital  
4  
5 175 contact, or on death. The survey was registered as a service evaluation project 9649 at Guy's  
6  
7 176 and St Thomas' NHS Trust, London. A subgroup from 43 cases with *SDHC* intragenic variants  
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10 177 had been published previously.<sup>11</sup>  
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### 14 179 **Clinical Surveillance**

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17 180 Participating centres reported clinical information: age and date of diagnosis, and if affected,  
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19 181 PPGL characteristics including size, anatomical location, malignancy (defined as the presence  
20  
21 182 of disease in a lymph node or more distant site where chromaffin cells are not present, i.e.  
22  
23 183 metastatic disease), required interventions and presence of other tumours. In the UK, prior to  
24  
25 184 March 2019, there was no consensus on the optimum surveillance programme for individuals  
26  
27 185 with *SDHx* germline variants, including *SDHC* for either affected individuals, or asymptomatic  
28  
29 186 carriers. Therefore, we asked centres to report on duration, interval between review and modes  
30  
31 187 of surveillance and screening outcomes. Standard biochemical tests differed by centre (24-hour  
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33 188 urine or plasma metadrenalines (MA), normetadrenaline (NMA) and/or 3-methoxytyramine  
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35 189 testing (3MT)).  
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### 42 191 **Genetic Testing**

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45 192 Genetic variants were identified using standard molecular techniques (Sanger sequencing,  
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47 193 multiplex ligase probe amplification or massively parallel sequencing via next generation  
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49 194 sequencing or exome sequencing). Germline variants were reported according to American  
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51 195 College of Medical Genetics and Genomics guidelines (ACMG).<sup>15</sup> Centres reported germline  
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53 196 variants, proband status, whether they were from a known *SDHC* kindred and additional PPGL  
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55 197 variants, if found. Reported variants were compared against variants in NIHR ClinVar  
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57 198 registries.<sup>16</sup>  
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## 200 **Structural Analysis and assessment of variant pathogenicity**

201 Structural integrity and succinate dehydrogenase (SDH) complex formation and structural  
202 prediction analyses were undertaken for all missense variants using IPS-3D, DUET and  
203 mCSM-PPI2.<sup>17-19</sup> These are reported alongside functional (mutPred) and pathogenicity scores,  
204 PolyPhen2 and SIFT.<sup>20-22</sup> Structural comparisons were performed using the porcine SDHC  
205 ortholog 1ZOY structure, with 91.7 % sequence homology, as no human SDHC structure is  
206 currently publicly available (Supplementary Figure 1).<sup>23</sup> Disease allele frequency was  
207 reviewed in gnomAD v3.1.1 database (<https://gnomad.broadinstitute.org/>).

208

## 209 **Data Analysis**

210 Data analysis and figure generation was undertaken using statistical computing language R  
211 version 4.0.3 (2020-10-10). Cumulative risk was estimated for pathogenic variants using  
212 survminer (ver. 0.4.8) and cmprsk (ver. 2.2-10) packages in individuals with likely pathogenic  
213 or pathogenic variants.

214

## 215 **Results**

### 216 **Demographic features**

217 A total of 91 *SDHC* variant cases from 18 UK centres were included. See Table 1. There were  
218 41 males (45%) and 50 females (55%), median age at genetic diagnosis 43 years (range: 11-  
219 79). Forty-six (51%) cases were probands, 45 (49%) were relatives identified through cascade  
220 testing from 22 kindreds. Fifty-one cases (56%) were disease-affected, 40 (44%) were  
221 unaffected. The 51 disease-affected cases median age at diagnosis was 43 years (range: 12-79).  
222 Unaffected cases (n=40) were younger with a median age at genetic diagnosis of 38 years

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223 (range: 11-79). Three non-probands had *SDHC*-associated disease (HNPGL (n=2) and PA  
224 (n=1)) diagnosed prior to predictive testing.

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### 226 **Clinical features**

227 There were 46 PPGL tumours diagnosed in 45 *SDHC* variant cases (Table 1), 65.2% were  
228 HNPGL, 6.5% thoracic PGL, 21.7% abdominal PGL and 6.5% PCC. HNPGLs were most  
229 frequent (n=30), located as follows: six carotid body (CBT), nine jugular, six vagal, one  
230 nasopharyngeal and eight HNPGL (site unspecified). Eleven cases (21.6%) developed *SDHC*-  
231 associated tumours: GIST (n=6, all female), Pituitary Adenoma (PA, n=3) and RCC (n=2). Of  
232 these, five cases presented with associated tumours, but no PPGL (GIST n=3, RCC n=1, PA  
233 n=1). Three GIST tumours showed SDHB immunonegativity and another was confirmed as  
234 wild-type. Eleven patients had multiple tumours: HNPGL and PA (n=2), HNPGL and thoracic  
235 PGL, thoracic PGL and adrenal adenoma, multiple RCCs, and GIST with either EAPGL (n=3),  
236 pulmonary chondroma (n=2) or oesophageal leiomyoma. Additional tumours include  
237 meningioma and bilateral breast cancer (see Supplementary Table 1 and Supplementary Table  
238 2).

239

240 Malignant disease was identified in 19.6% (9/46) PPGL cases (Table 1). Where tumour sizes  
241 were recorded, all malignant HNPGL were >50mm, including two vagal tumours. Two cases  
242 had malignant EAPGLs, the youngest with a 45mm tumour aged 12, another aged 30 with a  
243 78mm tumour, who died from metastatic disease aged 36. There were eight malignant *SDHC*-  
244 related tumours (six GISTs, two RCCs).

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246 In the disease-affected group, fifty interventions were recorded from 1977 to 2019 across 37  
247 cases (Table 1). Main treatment modalities included surgical resection (n=33), radiotherapy

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3 248 intervention (n=8) and active surveillance (n=13). Radiotherapy interventions included  
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5 249 external beam radiotherapy, stereotactic radiotherapy and gamma knife radiosurgery. Nine  
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8 250 HNPGL (30% of HNPGLs), two thoracic PGL, and two GIST cases remain on active  
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10 251 surveillance.  
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### 15 253 **Tumour incidence**

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17 254 Tumour incidence for probands with PPGL or GIST (n=37) and non-proband *SDHC*  
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19 255 pathogenic variant cases (n=45) is shown in Figure 1. *SDHC* tumour incidence at age 40 and  
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21 256 60 years was 40.5% and 94.6% in probands, and 4.4% and 6.7% in non-probands respectively.  
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24 257 Cumulative risk (95% CI) at age 20, 40 and 60 years was 0.05 (CI:0-0.12), 0.4 (CI:0.22-0.54)  
25  
26 258 and 0.94 (CI:0.79-0.99) in probands, and 0, 0.08 (CI:0-0.18) and 0.16 (CI:0-0.31) in non-  
27  
28 259 probands respectively (see Supplementary Figure 2). Age of diagnosis comparison between  
29  
30 260 probands and non-probands (unpaired Student's t-test with two-tailed p value) p value = 0.178,  
31  
32  
33 261 95% C.I. = 0 to 27.01. Difference between means (non-probands - probands)  $\pm$  SEM = 10.91  
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35 262  $\pm$  7.957.  
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### 40 264 **Surveillance**

41  
42 265 Screening information dates from 2006, with centres reporting imaging and biochemistry  
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44 266 according to local protocols, (biochemical monitoring and imaging every 12-36 months). See  
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46  
47 267 Table 2. Imaging modalities included whole body (WB-)MRI from skull base-pelvis,  
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49 268 Computed Tomography and abdominal Ultrasound scan. Information regarding  
50  
51 269 sequence/nature of imaging protocols was not gathered. Biochemical modalities included  
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54 270 either 24-hour urine or plasma metanephrines measurement. Chromogranin A data was not  
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56 271 collected unless additionally reported by the centre.  
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3 273 Across the whole cohort, 68 (75%) had at least two rounds of surveillance screening using  
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5 274 cross-sectional imaging, predominantly WB-MRI. Forty-two (46%) individuals had at least  
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8 275 two rounds of biochemical testing. Seven of 51 disease-affected cases (13.7%, two HNPGL,  
9  
10 276 five EAPGL) had raised biochemistry at diagnosis (elevated MA, or NMA or 3-MT). The  
11  
12 277 overall median length of surveillance was 42 months (3.5 years, range: 4-107 months).

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15 278  
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17 279 Surveillance data for 40 out of 45 non-proband asymptomatic individuals was available for a  
18  
19 280 median period of 45.5 months (3.8 years). Five individuals were awaiting screening. Two  
20  
21 281 individuals had tumours detected at baseline screen (5%), whereas 38 individuals had no  
22  
23 282 tumours identified (95%). Twenty individuals had at least two rounds of cross-sectional  
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25 283 imaging, 23 had at least two rounds of biochemical testing, without additional tumours  
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27 284 identified (Table 2).

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33 286 The eldest non-proband, diagnosed aged 79, had raised NMA and an EAPGL and GIST  
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35 287 identified on MRI. After EAPGL surgery, the individual had annual MRI surveillance, with  
36  
37 288 the GIST remaining stable. Another non-proband aged 60 had a CBT and thoracic PGL  
38  
39 289 diagnosed on MRI and fully characterised by <sup>18</sup>FDG-PET scan. The CBT was excised and the  
40  
41 290 thoracic PGL has been monitored by MRI. Their PGLs showed no malignant features, with  
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43 291 sizes smaller than average in this cohort: thoracic PGL 21mm; EAPGL 38mm (EAPGL and  
44  
45 292 PCC tumour size average  $45.5 \pm 25.1$ mm); HNPGL 22mm (HNPGL tumour size average  $32.9$   
46  
47  $\pm 16.9$ mm) (Supplementary Table 1 and Supplementary Table 2).

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49 293  
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51 294  
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53 295 Surveillance data for 46 proband individuals (following diagnostic investigations) was  
54  
55 296 available for a median period of 26 months (2.17 years, range: 4-107 months). Thirty-five  
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57 297 probands had at least one subsequent surveillance screen: 34 individuals had at least one screen  
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3 298 with cross-sectional imaging, 34 individuals had at least one screen including biochemical  
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5 299 testing. Twenty-four individuals underwent at least two rounds of cross-sectional imaging. No  
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8 300 new PPGLs were identified.  
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### 12 302 **Genetic Variants**

14 303 Twenty-four genetic *SDHC* variants were identified, including four single exon deletions, 19  
16 304 intragenic variants and one intronic variant distributed along the entire coding sequence of the  
18 305 gene (see Table 3 and Figure 2). Eighteen *SDHC* variants have previously been described. Six  
20 306 *SDHC* variants are novel: three exon deletions (exon 4 deletion, c.371\_372del. p.(Leu124fs),  
22 307 and exon 5 deletion), two missense variants (*SDHC*:c.200T>A, p.(Met67Lys) and  
24 308 *SDHC*:c.257G>A, p.(Gly86Asp)), and an intronic variant *SDHC*:c.(241+9)-(241+10)del.  
26 309

28 310

30 311 The 5'-UTR variant *SDHC*:c.-38G>A initially reported as a VUS, was reclassified as benign  
32 312 (Table 3). Three cases had additional *SDHx* variants, none classified as pathogenic. One  
34 313 individual with PCC aged 39 had two pathogenic variants, *SDHC*:c.148C>T, p.(Arg50Cys)  
36 314 and *RET*:c.2410G>A, p.(Val804Met). They underwent adrenalectomy and thyroidectomy,  
38 315 where no C-cell hyperplasia or medullary thyroid cancer was found. PCC occurs rarely in RET  
40 316 V804M cases.<sup>24</sup> LOH analysis of PCC tumour showed normal *SDHC* results, indicating RET  
42 317 V804M may have driven PCC development.  
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48 320 All 11 missense coding variants were assessed according to ACMG guidelines, five (45.4%)  
50 321 met criteria for likely pathogenic variants, whilst five (45.4%) are VUSs and one is considered  
52 322 likely benign (Table 4). Overall, 16 out of 24 *SDHC* variants identified are considered  
54 323 pathogenic or likely pathogenic, six are VUSs and two are benign. Genotype-phenotype  
56 324 correlations are included in the appendix.  
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**324 Structure-phenotype correlation**

325 All missense variants are predicted to have a destabilising effect on SDHC complex formation  
326 (Table 4). The two novel variants *SDHC*:c.200T>A, p.(Met67Lys) and *SDHC*:c.257G>A,  
327 p.(Gly86Asp) are predicted to destabilise protein structure and SDH complex formation. Both  
328 variants are predicted to be ‘damaging’ and ‘pathogenic’ by PolyPhen2 and SIFT scores.  
329 HNPGL tissue from c.257G>A, p.(Gly86Asp) showed SDHB immunonegativity. The  
330 missense mutation most disruptive to complex formation (c.148C>T, p.(Arg50Cys)) disrupts  
331 the N-terminus flexible loop, which is critical for the for both the assembly and stability of  
332 Complex II.<sup>23</sup> The most destabilising missense mutation (c.377A>G, p.(Tyr126Cys)) results in  
333 a switch of bulky, hydrophobic tyrosine for a polar cysteine and interrupts strong stacking  
334 interactions within a hydrophobic pocket, which are important for the stability of the helical  
335 membrane anchor.<sup>23</sup> The two missense mutations predicted to not affect protein structure  
336 stability (c.214C>T, p.(Arg72Cys) and c.380A>G, p.(His127Arg)) both correspond to heme  
337 complex binding residues, with the latter being directly coordinated to the iron centre. The fact  
338 that the variant residues retain a degree of polarity, and that other coordinating residues remain  
339 intact, may explain the relative redundancy in protein stability in these variants. One variant  
340 *SDHC*:c.490A>T, p.(Met164Leu) was predicted to have a stabilising effect on protein  
341 structure, predicted to be ‘tolerated’/‘benign’ by structural analysis and classified as ‘likely  
342 benign’ by ACMG guidelines. In this case, the PCC tumour showed SDHB immunopositivity.

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**344 Discussion**

345 This multi-centre, nationwide UK case-series describes the largest *SDHC* cohort to date. The  
346 median age of genetic diagnosis for disease-affected cases was 43 years (range: 12-79), similar

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3 347 to that reported by Schiavi *et al.* (median 46 years, range: 13-73), younger than cases with  
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5 348 sporadic HNPGL (median 53 years, range: 15-83).<sup>13</sup>  
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10 350 As reported previously, we recorded a wide spectrum of tumours in *SDHC*-associated  
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12 351 disease.<sup>11</sup> We confirm the majority of *SDHC* cases develop isolated HNPGL (65.2%), with  
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14 352 EAPGL in 28.2%, and PCC in 6.5%. HNPGL are more common in *SDHD* (85%) than *SDHC*  
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16 353 but less common in *SDHB* (35%).<sup>9</sup> Previous studies reported 36% *SDHC* cases had multiple  
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18 354 PPGLs, however only one UK *SDHC* case had multiple PPGLs.<sup>9</sup> While *SDHC* cases have been  
19  
20 355 described as developing benign HNPGLs, six cases developed malignant HNPGLs (20%),  
21  
22 356 >50mm at diagnosis.<sup>13,25</sup> Two metastatic EAPGL cases presented young (12 and 30 years),  
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24 357 with tumours >45mm. These features are all predictors of aggressive disease. In a recent meta-  
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26 358 analysis, patients with *SDHC* tumours were reported to have a metastatic risk of 23% (CI: 0.10-  
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28 359 0.45) over an averaged follow-up period of 4.8 years.<sup>26</sup> Our marginally lower overall metastatic  
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30 360 PPGL rate of 19.6%, may reflect our slightly shorter follow-up period, averaged at 3.1 years.  
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32 361 The risk of malignant disease at 19.6% remains lower than the pooled risk in *SDHB* (31%), but  
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34 362 higher than in *SDHD* (8%).<sup>26</sup>  
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42 364 Our case series identified rarer tumours associated with *SDHC*: GIST, PA and RCC in 21.5%  
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44 365 of cases.<sup>27</sup> GIST was the second most common tumour after PPGL. Interestingly, five of the  
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46 366 six cases with GIST had related tumours, e.g. EAPGL (confirming Carney-Stratakis  
47  
48 367 syndrome), or pulmonary chondroma and oesophageal leiomyoma, evidence of incomplete  
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50 368 Carney's Triad. Unfortunately, there was no *SDHB* IHC testing in RCC or PA cases, so *SDH*-  
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52 369 deficiency remains unconfirmed. Whilst PA have been reported in *SDHC* cases, they occur  
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54 370 commonly in the general population at a frequency of 1 in 1,064 cases.<sup>28</sup> The association of  
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3 371 pituitary tumours as a feature of *SDHC*-related disease may therefore represent an incidental  
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5 372 finding.  
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10 374 We describe six novel germline *SDHC* variants: Three pathogenic exon deletions, two missense  
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12 375 VUS and one intronic variant. *In silico* structural analyses of all missense variants correlated  
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14 376 with pathogenicity classification for previously identified variants and demonstrated disruption  
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16 377 of key structural and functional elements of the SDH complex. Structural analyses predict  
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18 378 novel variants *SDHC*:c.200T>A, p.(Met67Lys) and *SDHC*:c.257G>A, p.(Gly86Asp) to be  
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20 379 pathogenic, correlating with SDHB immunonegativity in the *SDHC*:c.257G>A, p.(Gly86Asp)  
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22 380 case.  
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28 382 Reclassification of variants is a dynamic process and can have important implications for  
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30 383 clinical practice. One patient with an EAPGL has the non-coding variant *SDHC*:c.-38>A,  
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32 384 designated a VUS in 2008, but reclassified as a benign variant in 2018. *SDHC*:c.148C>T,  
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34 385 p.(Arg50Cys), and c.377A>G, p.(Tyr126Cys) were initially reported as VUSs, but recently  
35  
36 386 reclassified (in 2018 and 2021) as 'likely pathogenic', affecting nine probands. Reclassification  
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38 387 events are increasing, with one commercial laboratory reporting 7.7% of unique VUS being  
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40 388 reclassified over 10 years.<sup>29</sup> Upgrades from a VUS to a pathogenic variant will have clinical  
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42 389 consequences. Downgrades may be helpful in releasing patients from unnecessary surveillance.  
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44 390 Overall, 16 out of 24 *SDHC* variants identified are currently considered pathogenic or likely  
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46 391 pathogenic, with six remaining as VUSs and two now considered benign.  
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52 393 Screening outcomes were reported for all *SDHC* variant cases. Relatively few cases with  
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54 394 disease had a positive biochemical result (13.7%). This is unsurprising, reflecting the  
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56 395 propensity for *SDHC* patients to develop HNPGL, which are often non-secretory, underlining  
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3 396 the importance of imaging in PPGL detection. During follow-up, no metachronous tumours  
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5 397 were identified in probands, but tumour growth and progression of metastatic disease was  
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8 398 observed.

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12 400 All tumours found in asymptomatic non-proband cases were identified at initial work-up: three  
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14 401 PGLs and a GIST in two cases, detected by MRI. Reassuringly their PGL tumour sizes were  
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17 402 smaller than the averages across the cohort, indicating surveillance had detected disease earlier,  
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19 403 with possible beneficial outcomes. However, with only one GIST case in non-probands, the  
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21 404 penetrance of this tumour remains unclear. The range of treatments employed reflects the  
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23 405 changing practice for treating HNPGL disease. HNPGLs are slow-growing tumours with a  
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25 406 doubling time of 5.8 years.<sup>30</sup> Many patients with HNPGLs are now being considered for  
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28 407 nonsurgical therapy instead of operative intervention, which risks increased morbidity to  
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30 408 achieve surgical cure.<sup>31</sup> The broadened range of radiation techniques available and their  
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33 409 success, for example ablative stereo-tactic radiosurgery, has led to increased use of these  
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35 410 interventions. Discussion via a National MDT for managing HNPGLs in *SDHC*, in view of the  
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37 411 20% HNPGL malignancy risk, could provide a mechanism to enhance practice and outcomes.

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42 413 This study reports *SDHC* tumour incidence at age 40 and 60 years was 40.5% and 94.6% in  
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44 414 probands, and 4.4% and 6.7% in non-probands respectively. This is in keeping with the  
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46 415 published findings of Benn *et al.*, who report predicted lifetime penetrance of 8.3% (95% CI  
47  
48 416 3.5% to 18.5%), among patients selected for known *SDHC* pathogenic or likely pathogenic  
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50 417 variants.<sup>32</sup> This study highlights low cumulative tumour risk in non-probands, 16% (95% CI:0-  
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52 418 0.31) at 60 years compared to a previous estimate of 25% (95% CI 0-0.57).<sup>11</sup> This may reflect  
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54 419 the younger average age of non-probands at 38 years, compared to the average age of affected  
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56 420 cases at 43, with tumours yet to develop in non-probands. Certainly, for proband cases, tumours  
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3 421 presented throughout life and metastatic disease was seen at all ages, emphasizing lifelong  
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5 422 tumour susceptibility.

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10 424 The aim of any surveillance programme in *SDHC* patients at risk of PPGL, GIST and RCC is  
11  
12 425 to detect early disease and reduce the chance of metastatic spread.<sup>11</sup> However, with low tumour  
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14 426 incidence in non-probands and lower metachronous tumour risk in probands than previously  
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16 427 documented, surveillance frequency might safely be lengthened from recommended biennial  
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18 428 cross-sectional imaging, thus reducing costs and the burden to patients of frequent scans.<sup>7</sup>  
19  
20 429 Recent screening studies also confirm low tumour rates in asymptomatic *SDHC* carriers with  
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22 430 4% (1/28) having PPGL diagnosed at baseline,<sup>33</sup> matching our experience of 5% (2/40) cases  
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24 431 identified.

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30 433 In 2016, the European Society for Endocrinology published a clinical practice guideline for  
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32 434 patients operated on for PPGL tumours, including any genetic variants. It recommended  
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34 435 assaying plasma or urinary MN and 3MT every year, and performing imaging tests every 1-2  
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36 436 years in patients with biochemically inactive PPGL, to screen for local or metastatic recurrence  
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38 437 or new tumours.<sup>34</sup> While this has been broadly adopted for patients with other *SDHx* variants  
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40 438 (i.e. *SDHB*)<sup>9</sup>, evidence from our surveillance data suggests this was not routinely achieved for  
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42 439 *SDHC* cases across the UK prior to 2020. Following the results of this study, we recommend:

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47 440 1. Lifelong tumour specific follow-up (to monitor recurrent or metastatic disease, as  
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49 441 determined by specialist multidisciplinary teams) for affected patients.
- 50  
51 442 2. For metachronous PPGL and RCC detection, annual clinical review with plasma  
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53 443 metanephrines, and WB-MRI (skull base, neck, thorax, abdomen and pelvis) 3-5 yearly.
- 54  
55 444 To identify GISTs, gastric symptoms should be enquired of at clinical review and a full  
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57 445 blood count (FBC) measurement taken to assess for anaemia. For patients with  
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3 446 suggestive symptoms or anaemia, Oesophageal-Gastro-Duodenoscopy (OGD) should  
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5 447 be considered.

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8 448 3. Non-probands commence annual clinical review with biochemistry and FBC from 10  
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10 449 years, and WB-MRI, as above, from 15 years.

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12 450 4. Patients with a *SDHC* VUS and PPGL continue tumour specific follow-up. For their  
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14 451 potential metachronous tumour risk, annual clinical review with biochemistry and FBC  
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16 452 for 10 years, and WB-MRI, as above. Assessment of *SDHC* VUS status to determine if  
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18 453 lifelong WB-MRI surveillance is indicated after 10 years (provided there are no high-  
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20 454 risk features, e.g. multiple PPGLs or familial disease).

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24 455 5. *SDHB* immunohistochemistry, molecular analysis (LOH studies or somatic testing),  
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26 456 histopathological review and consideration of tumour methylome and metabolomics  
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28 457 profiling in PPGL and additional tumours, to aid variant classification and determine  
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30 458 whether tumours are truly *SDHC*-related.<sup>27</sup>

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35 460 Despite this study being the largest *SDHC* patient cohort reported, we recognise its limitations,  
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37 461 including retrospective data collection, incomplete clinical information and the challenge of  
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39 462 case ascertainment. Our short follow-up period may not have captured all tumour occurrences  
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41 463 or metastatic disease and the small numbers in the cohort may have insufficient power for some  
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43 464 differences to be realised. At present, there is no UK register for patients with *SDHx* disease,  
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45 465 their surveillance outcomes or impact of surveillance on morbidity and mortality. Such a  
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47 466 register would also benefit from access to centralised genomics data and cancer registration,  
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49 467 which would provide a richer overview of *SDHC*-associated disease.

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56 469 In summary, this UK multi-centre study reports the largest dataset of 91 affected and unaffected  
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58 470 *SDHC* germline variant carriers to date, with six novel *SDHC* variants out of 24 identified. The  
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3 471 clinical manifestations of disease in this cohort have increased our understanding of *SDHC*  
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5 472 disease and demonstrate the importance of lifelong surveillance of these patients, who have a  
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7 473 1/5 risk of metastatic disease. It is imperative that prospective data is collected to help tailor  
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9 474 surveillance programmes for the benefit of asymptomatic *SDHC* patients and to provide  
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11 475 accurate information for genetic counselling of patients about their lifetime risk of disease.  
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49 589 **Tables (included as editable excel files within the submission)**

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52 591 **Table 1: Clinical characteristics of 91 SDHC variant cases, tumour diagnoses, treatments**  
53 **and outcomes.**  
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58 594 **Table 1:**  
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3 595 <sup>a</sup> Other includes skull base, glomus, HNPGL site not clearly specified.  
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5 596 <sup>b</sup> SDHB IHC or LOH studies were not performed on PA or RCC tumours.  
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7 597 <sup>c</sup> Some cases had multiple tumours - see Supplementary Table 1.  
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10 598  
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12 599 **Table 2: Screening modalities and results, for proband and non-proband individuals.**  
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14 600 **Table 2:**  
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17 601 <sup>a</sup> Definitions of disease status: Positive indicates new diagnosis or new focus of metastatic  
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19 602 disease. Stable indicates radiologically stable disease. Negative indicates no evidence of  
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21 603 disease in screen, whether biochemically or radiologically.  
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24 604 <sup>b</sup> See main text for description of imaging and biochemical modalities. For non-probands,  
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26 605 investigations undertaken within 6 months are considered within a single screen.  
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28 606 <sup>c</sup> One patient had undergone screening in 2012, but results were not available.  
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31 607 <sup>d</sup> Other forms of investigation included: Computed Tomography with biochemical testing  
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33 608 (CT/B), Bronchoscopy (Br), I123/I131 metaiodobenzylguanidine (MIBG) imaging with MRI  
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35 609 (MIBG/MRI) or Ultrasound scan with biochemical testing (US/B) and with MRI  
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37 610 (US/B/MRI).  
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42 612 **Table 3: List of all intragenic or regulatory site variants in *SDHC* [reference sequence**  
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44 613 **NM\_003001.3].**  
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47 614 **Table 3:**  
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49 615 Additional co-variants in *SDHB*, *SDHD* have also been annotated. Variants listed as ‘novel’,  
50  
51 616 have, to the best of our knowledge, not been published elsewhere.  
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53  
54 617 <sup>a</sup> One patient with *SDHC* c.148C>T variant has a concurrent pathogenic low penetrance *RET*  
55  
56 618 protooncogene variant, c.2410G>A, p.(Val804Met).  
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58 619 <sup>b</sup> This is an intronic variant. Its possible effect on splicing has not been investigated further.  
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621 **Table 4: Structural and pathogenicity prediction scores for all *SDHC* missense variants**  
622 **described in this cohort.**

623 **Table 4:**624 <sup>a</sup> The coexisting *SDHD* variant c.34G>A is registered on ClinVar as likely benign.625 <sup>b</sup> The coexisting *SDHB* variant c.8C>G is registered on ClinVar as a benign variant.626 <sup>c</sup> The coexisting *SDHD* variant c.319C>T is registered on ClinVar as a VUS.627 <sup>d</sup> Allele frequencies are as reported in gnomAD v3.1 (accessed via628 <https://gnomad.broadinstitute.org/> Nov 2020). Variants reported from position chr

629 1.161328466.

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631 **Figure Legends (Figures included as separate files within the submission)**

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633 **Figure 1: Tumour incidence of *SDHC*-associated disease across proband and non-**  
634 **proband groups.**

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636 **Figure 2: Lollipop plot depicting the distribution of *SDHC* variants.** Diagram illustrating  
637 71 germline *SDHC* variants along the amino acid sequence, colour-coded by the mutation class:  
638 start loss (11), truncating (24) and missense (36) genetic variants. The Y axis represents the  
639 number of occurrences for each *SDHC* variant in our cohort. The variant *SDHC*:c.397C>T,  
640 p.(Arg133Ter) is the most prevalent variant displayed, occurring 14 times.

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642 **Supporting information and Appendix (Supporting tables and figures included as**  
643 **editable excel files within the submission)**

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3 6444  
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6 645 **Supporting information:**7  
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9 646 **[1] Supplementary Table 1: Patients with multiple *SDHC*-related tumours and their**  
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11 647 **outcomes.**12  
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14 648 **Supplementary Table 1:** Abbreviations: NR = No Record. GIST= Gastrointestinal tumour.15  
16 649 CBT= carotid body tumour. All patients with GIST are female, median age 33 (range 22-79)17  
18 650 years. <sup>a</sup> HNPGL average size across cohort  $32.9 \pm 16.9$ mm, EAPGL/PCC average size across19  
20 651 cohort  $45.5 \pm 25.1$ mm.  
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24 65225  
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27 653 **[2] Supplementary Table 2: Tumours in Non-proband *SDHC* cases.**28  
29  
30 654 **Supplementary Table 2:** NR = No record. HNPGL average size across cohort  $32.9 \pm$ 31  
32 655  $16.9$ mm, EAPGL/PCC average size across cohort  $45.5 \pm 25.1$ mm.  
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35 65636  
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38 657 **[3] Supplementary Table 3: Clinical characteristics and disease phenotypes listed by**  
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40 658 ***SDHC* variant.**41  
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43 659 **Supplementary Table 3:** Cases with \* have malignant disease. <sup>a</sup> Cases are separated by44  
45 660 commas. <sup>b</sup> One patient with *SDHC* c.148C>T, p.(Arg50Cys) variant has a concurrent46  
47 661 pathogenic low penetrance *RET* protooncogene variant, c.2410G>A, p.(Val804Met).48  
49 662 Abbreviations: HN HNPGL unknown site, LCBT left carotid body tumour, RCBT right50  
51 663 carotid body tumour, L left glomus, RG right glomus, LJ left jugulare, RJ right jugulare, LV52  
53 664 left vagus, RV right vagus, V vagus (site unspecified), NPG nasopharyngeal, LSB left skull54  
55 665 base, GIST gastrointestinal stromal tumour, RCC renal cell cancer, PA pituitary adenoma, <sup>c</sup>56  
57 666 Pituitary adenomas were prolactinomas.  
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3 667 **[4] Supplementary Figure 1:** BLAST alignment of Human Succinate dehydrogenase  
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5 668 cytochrome b560 subunit, SDHC (Q99643) and Pig SDHC (D0VWV4).<sup>35</sup> Structural prediction  
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7 669 analyses were based on the high-resolution 1ZOY structure of succinate dehydrogenase from  
8  
9 670 *Sus scrofa* (<https://www.rcsb.org/structure/1zoy>, PMID: 15989954).

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16 672 **[5] Supplementary Figure 2:** Cumulative tumour risk and 95% CI for probands and non-  
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18 673 probands with pathogenic variants in *SDHC* with median ages for both groups highlighted. NB:  
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20 674 Due to limited number of older non-probands, the final value of the corresponding line is likely  
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22 675 to be over-inflated (95% CI: 0-89.6%).

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## 677 **Appendix**

31 678 **[1] Supplementary information regarding centres approached.** An invite was initially sent  
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33 679 to 23 genetics centres in the UK and these departments linked us to relevant endocrine services  
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35 680 involved in the care of their *SDHC* families. Genetic centres in Aberdeen, Bristol, Dundee,  
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37 681 Exeter, St George's Hospital (SW London) and University Hospital Southampton NHS Trust,  
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39 682 Wessex did not have any known *SDHC* variant cases. The All Wales Genetic service was able  
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41 683 to identify *SDHC* cases, but unable to provide any clinical information due to difficulties in  
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43 684 locating information from patient records. There were 28 cases from London centres (Guy's  
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45 685 and St Thomas' NHS Trust, King's College Hospital NHS Foundation Trust, Bart's Health  
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47 686 NHS Foundation Trust, North West London Genetic Service, including Imperial College  
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49 687 Healthcare Foundation NHS Trust and University College London Hospital NHS Foundation  
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51 688 Trust). Outside of London, there were 63 cases in total from the following centres: Oxford  
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53 689 University Hospital NHS Foundation Trust, Cambridge University Hospital Foundation NHS  
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55 690 Trust, University Hospitals Birmingham NHS Trust, Nottingham University Hospitals NHS  
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3 691 Trust, Sheffield Teaching Hospitals NHS Foundation Trust, Leeds Teaching Hospitals NHS  
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5 692 Trust, University Hospitals of Leicester, Manchester University NHS Trust, Newcastle Upon  
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8 693 Tyne Hospitals NHS Foundation Trust, Edinburgh Royal Infirmary, Glasgow Royal Infirmary  
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10 694 and Belfast City Hospital, Northern Ireland. The largest contributing centre in London was  
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12 695 GSTT and outside of London was Newcastle.

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15 696 **[2] Supplementary information regarding genotype-phenotype evaluation.** Bayley *et al.*  
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17 697 reported that truncating *SDHB* and *SDHD* variants were significantly over-represented in  
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19 698 affected cases, with *SDHB/D* truncations associated with higher risk of developing PPGLs,  
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21 699 compared with missense variants.<sup>36</sup> In our *SDHC* cohort, truncating variants were not  
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23 700 associated with PPGL over other disease sites (two-sided Fischer's exact test,  $p = 0.999$ , CI:  
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25 701 0.246-4.028), malignant disease ( $p=0.999$ , CI: 0.232-5.080), or development of non-PGL  
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27 702 malignancy (GIST and RCC,  $p= 0.683$ , CI: 0.069-3.637) (Supplementary Table 3). Given the  
28  
29 703 small sample size per variant, it is not meaningful to associate predicted pathogenicity with  
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31 704 tumour incidence in this cohort, although this has been demonstrated in *SDHB* and *SDHD*  
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33 705 variants.<sup>11</sup>

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**Table 1: Clinical characteristics of 91 SDHC variant cases, tumour diagnoses, treatments and outcomes**

Characteristics	SDHC cases
Male:Female	41:50
Median age at genetic diagnosis (years, range)	43 (11-79)
Median age at tumour diagnosis (years, range)	43 (12-79)
Total number of cases affected by PPGL or SDHC-related tumours	51
Number of tumours and site (n, %)	
Total PPGL tumours	46
Total HNPGL tumours and site	30 (65.2%)
Carotid Body Tumour	6
Jugular	9
Vagal	6
Nasopharyngeal	1
Other <sup>a</sup>	8
Metastatic HNPGL disease (n, %)	6 (20%)
HNPGL Tumour size (mm)	32.9 ± 16.9
Number of EAPGL tumours and site (n, %)	13 (28.2%)
Thoracic PGL	3 (6.5%)
Abdominal and retroperitoneal PGL	10 (21.7%)
Metastatic EAPGL	2 (4.3%)
Phaeochromocytomas (PCC)	3 (6.5%)
Metastatic PCC	1 (33%)
EAPGL/PCC Tumour size (mm)	45.5 ± 25.1
Patients with multiple PPGL	1
Total metastatic PPGL disease (n, %)	9 (19.6%)
Type of metastases	
Lymph node	3
Bone	4
Liver	2
Lung	1
SDHC-related tumours <sup>b</sup>	11
GIST	6
Pituitary Adenoma (PA)	3
Renal Cell Cancer (RCC)	2
Total malignant SDHC-related tumours	8 (72.7%)
Total number of tumours in SDHC cases <sup>c</sup>	57
Intervention/active surveillance	
Pre-operative embolisation	3
Surgery	33
Radiotherapy	8
MIBG	3
Radionuclide therapy (Yttrium <sup>90</sup> )	1
Targeted therapy -Imatinib +/- Sunitinib (GIST treatment)	2
HNPGL - on active surveillance	9
Thoracic PGL - on active surveillance	2
GIST - on active surveillance	2
PPGL deaths	1

**Table 1:** <sup>a</sup> Other includes skull base, glomus, HNPGL site not clearly specified.

<sup>b</sup> SDHB IHC or LOH studies were not performed on PA or RCC tumours.

<sup>c</sup> Some cases had multiple tumours - see Supplementary Table 1.

**Table 2: Screening modalities and results, for proband and non-proband individuals**

Group	First screen	Second screen	Third screen	Fourth Screen	Fifth Screen	Total surveillance
<b>Non-proband</b>	<b>Total (n = 45)</b>	<b>45</b>	<b>→ 34</b>	<b>→ 26</b>	<b>→ 20</b>	<b>→ 16</b>
	Positive <sup>a</sup>	2	0	0	0	0
	Stable	0	2	2	2	2
	Negative	38	23	17	13	9
	Not available/pending	5	9	7	5	5
	MRI and biochemistry <sup>b</sup>	35	9	11	11	7
	MRI alone	3	3	2	1	0
	Biochemistry alone	1	14	5	1	4
	Not available/pending	4	5	5	7	5
	Other <sup>d</sup>	2	3	3	0	0
	Other screening modalities (per case) <sup>d</sup>	US/B, US/B/MRI	US/B, US/B, US/B	CT, US/B, US/B		
	Median interval (months, range)		16.5 (11-27)	12 (8-26)	16 (9-32)	15 (12-63)
	Median surveillance duration (months, range)					45.5 (12-103)
<b>Proband</b>	<b>Total (n = 46)</b>	<b>46</b>	<b>→ 35</b>	<b>→ 27</b>	<b>→ 22</b>	<b>→ 15</b>
	Positive <sup>a</sup>	14	5	1	1	1
	Stable	1	3	5	3	2
	Negative	19	15	12	10	4
	Not available/pending <sup>b</sup>	12	12	9	8	8
	MRI and biochemistry	23	15	13	8	3
	MRI alone	7	4	3	5	3
	Biochemistry alone	1	6	3	1	1
	Not available/pending	11	8	8	8	8
	Other <sup>d</sup>	4	2	0	0	0
	Other screening modalities <sup>d</sup>	CT/MRI, Br, MIBG/MRI, CT/B	CT/B, Br			
	Median interval (months, range)		12 (4-78)	12 (4-29)	12 (1-31)	11 (2-14)
	Median surveillance duration (months, range)					26 (4-107)

**Table 2:** <sup>a</sup> Definitions of disease status: Positive indicates new diagnosis or new focus of metastatic disease. Stable indicates radiologically stable disease. Negative indicates no evidence of disease in screen, whether biochemically or radiologically.

<sup>b</sup> See main text for description of imaging and biochemical modalities. For non-probands, investigations undertaken within 6 months are considered within a single screen.

<sup>c</sup> One patient had undergone screening in 2012, but results were not available.

<sup>d</sup> Other forms of investigation included: Computed Tomography with biochemical testing (CT/B), Bronchoscopy (Br), <sup>123</sup>I/<sup>131</sup>I metaiodobenzylguanidine (MIBG) imaging with MRI (MIBG/MRI) or Ultrasound scan with biochemical testing (US/B) and with MRI (US/B/MRI).

**Table 3: List of all intragenic or regulatory site variants in SDHC [reference sequence NM\_003001.3].**

Gene	SDHC Exon	Variant SDHC/SDHx	Result SDHC/SDHx	Protein change SDHC/SDHx	Number of probands	Number of cases	ClinVar ID or reference	Reported effect SDHC/SDHx	Allele frequency <sup>c</sup>
SDHC	Promoter 1 (-30)	c.-38G>A	Does not affect function	p.=	1	1	368837	Benign	Not described
		c.1A>G	Start codon lost	p.(Met1?)	3	4	407060	Pathogenic	1.32E-05
		c.1A>T	Start codon lost	p.(Met1?)	2	7	653751	Pathogenic	Not described
	2 (21)	Exon 2 deletion	Copy Number Variant	p.?	1	1	Burnichon <i>et al.</i> 2009.	Pathogenic	Not described
		c.43C>T	Stop gain	p.(Arg15Ter)	2	7	41776	Pathogenic	1.97E-05
	3 (78)	c.78-1G>A	Acceptor splice site variant	p.=	1	1	185473	Likely Pathogenic	6.57E-06
		c.148C>T <sup>a</sup>	Missense variant	p.(Arg50Cys)	6	12	135194	Likely Pathogenic	6.57E-06
		c.148C>T and SDHD c.34G>A	Missense variant/Missense variant	p.(Arg50Cys)/p.(Gly12Ser)	1	1	135194; 6895	Likely Pathogenic and Likely benign	6.57E-06
		c.164A>G	Missense variant	p.(His55Arg)	1	1	239449	VUS	6.57E-06
	4 (180)	Exon 4 deletion c.(179+92_180-1)_(241+107_242-1)del	Copy Number Variant	p.?	1	1	<b>Novel</b>	<b>Novel Pathogenic</b>	Not described
		c.200T>A	Missense variant	p.(Met67Lys)	1	1	<b>Novel</b>	<b>Novel VUS</b>	Not described
		c.214C>T	Missense variant	p.(Arg72Cys)	0	2	653952	Likely Pathogenic	1.31E-05
		c.215G>A	Missense variant	p.(Arg72His)	2	8	407044	VUS	Not described
		c.224G>A and SDHB c.8C>G	Missense variant/Missense variant	p.(Gly75Asp)/p.(Ala3Gly)	1	1	189841; 12791	Conflicting (Likely Pathogenic/VUS) and Benign	Not described
		c.(241+9)_(241+10)del <sup>b</sup>	Unknown	p.=	1	1	<b>Novel</b>	<b>Novel VUS</b>	Not described
	5 (242)	Exon 5 deletion	Copy Number Variant	p.?	1	1	<b>Novel</b>	<b>Novel Pathogenic</b>	Not described
		c.257G>A	Missense variant	p.(Gly86Asp)	1	1	<b>Novel</b>	<b>Novel VUS</b>	Not described
		c.263C>T	Missense variant	p.(Ser88Leu)	1	1	407056	VUS	1.31E-05
		c.345dupA	Frame-shift elongation	p.(Ala116SerfsTer2)	1	1	Andrews <i>et al.</i> 2018	Pathogenic	Not described
		c.371_372del	Frame-shift elongation	p.(Leu124fs)	1	2	<b>Novel</b>	<b>Novel Pathogenic</b>	Not described
		c.377A>G	Missense variant	p.(Tyr126Cys)	1	1	428933	Likely pathogenic	2.63E-05
		c.377A>G and SDHD c.319C>T	Missense variant/Missense variant	p.(Tyr126Cys)/p.(Leu107Phe)	1	1	428933; 578681	Likely pathogenic and VUS	2.63E-05
		c.380A>G	Missense variant	p.(His127Arg)	3	5	187084	Pathogenic	6.57E-06
		c.397C>T	Stop gain	p.(Arg133Ter)	7	14	183753	Pathogenic	6.58E-06
	6 (406)	Exon 6 deletion	Copy Number Variant	p.?	4	14	Andrews <i>et al.</i> 2018	Pathogenic	Not described
		c.490A>T	Missense variant	p.(Met164Leu)	1	1	184146	Conflicting (Likely Benign/VUS)	1.86E-04
TOTAL					46	91			

**Table 3:** Additional co-variants in SDHB, SDHD have also been annotated. Variants listed as 'novel', have, to the best of our knowledge, not been published elsewhere.

<sup>a</sup> One patient with c.148C>T variant has a concurrent pathogenic low penetrance *RET* protooncogene variant, c.2410G>A, p.(Val804Met).

<sup>b</sup> This is an intronic variant. Its possible effect on splicing has not been investigated further.

<sup>c</sup> Allele frequencies are as reported in gnomAD v3.1.1 (accessed via <https://gnomad.broadinstitute.org/> on 01/07/2021).

**Table 4: Structural and pathogenicity prediction scores for all *SDHC* missense variants described in this cohort**

<i>SDHC</i> Exon	<i>SDHC</i> Variant	Allele frequency <sup>d</sup>	INPS-3D Kcal/mol	mCSM-PP12 Kcal/mol	DUET Kcal/mol	mutPred	PolyPhen HVAR.rank	SIFT	Predicted pathogenicity	Effect as per ACMG guidelines	Evidence
3 (78)	c.148C>T <sup>a</sup>	6.57E-06	-0.297	-1.926	-2.101	0.78	0.88582	Damaging	Pathogenic	Likely pathogenic	PS3_Moderate, PS4_Moderate, PM2, PP3
	c.164A>G	6.57E-06	-0.532	-0.141	-1.456	0.933	0.84481	Damaging	Pathogenic	VUS - not enough evidence	PS4_Moderate, PM2, PP3
4 (180)	c.200T>A	<i>Not described</i>	-1.277	-0.256	-0.546	0.909	0.67921	Damaging	Pathogenic	VUS - not enough evidence	PM2, PP3
	c.214C>T	1.31E-05	0.146	-1.001	-0.611	0.89	0.86255	Damaging	Pathogenic	Likely Pathogenic	PS3_Moderate, PS4_Moderate, PM1_Supporting, PM2, PP3
	c.215G>A	<i>Not described</i>	-0.043	-0.558	-1.517	0.877	0.86255	Damaging	Pathogenic	Likely Pathogenic	PS4_Moderate, PM2, PM5, PP3
	c.224G>A <sup>b</sup>	<i>Not described</i>	-0.486	-0.058	-1.354	0.943	0.92359	Damaging	Pathogenic	VUS - not enough evidence	PS4_Moderate, PM2, PP3
5 (242)	c.257G>A	<i>Not described</i>	-0.164	-2.107	-2.245	0.79	0.995	Damaging	Pathogenic	VUS - not enough evidence	PM2, PP3
	c.263C>T	1.31E-05	-0.083	-0.23	0.452	0.552	0.087	Tolerated	Pathogenic	VUS - not enough evidence	BP4
	c.377A>G <sup>c</sup>	2.63E-05	-1.939	-0.563	-2.138	0.823	0.92359	Damaging	Pathogenic	Likely pathogenic	PS4_Moderate, PM1_Supporting, PM2, PP3
	c.380A>G	6.57E-06	0.873	-0.285	-1.309	0.945	0.97372	Damaging	Pathogenic	Likely pathogenic	PS4_Moderate, PM2, PP4, PP3
6 (406)	c.490A>T	1.86E-04	-0.179	-0.15	0.207	0.476	0.01387	Tolerated	Benign	Likely benign	PS4, BP4

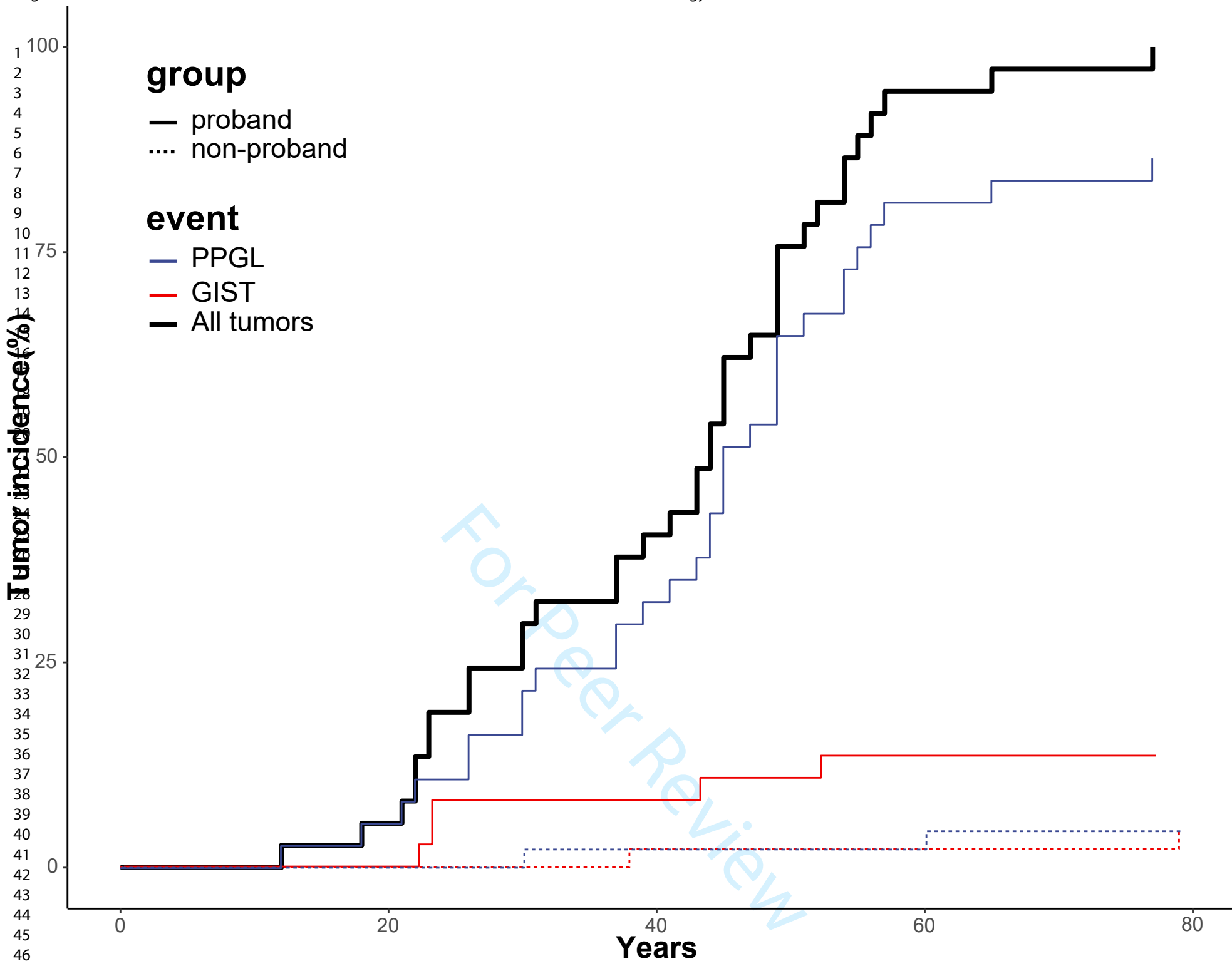
**Table 4:** Variants reported from position chr 1.161328466.

<sup>a</sup> The coexisting *SDHD* variant c.34G>A is registered on ClinVar as likely benign.

<sup>b</sup> The coexisting *SDHB* variant c.8C>G is registered on ClinVar as a benign variant.

<sup>c</sup> The coexisting *SDHD* variant c.319C>T is registered on ClinVar as a VUS.

<sup>d</sup> Allele frequencies are as reported in gnomAD v3.1.1 (accessed via <https://gnomad.broadinstitute.org/> on 01/07/2021).



For Peer Review

**Probands: Number at risk (number censored)**

<b>All</b>	37 (0)	35 (0)	22 (0)	2 (0)	0 (0)
<b>PPGL</b>	37 (0)	35 (0)	22 (3)	2 (5)	0 (5)
<b>GIST</b>	37 (0)	35 (2)	22 (12)	2 (30)	0 (32)
	0	20	40	60	80

**Non-probands: Number at risk (number censored)**

<b>All</b>	45 (0)	35 (10)	21 (22)	12 (31)	0 (41)
<b>PPGL</b>	45 (0)	35 (10)	21 (23)	12 (32)	0 (43)
<b>GIST</b>	45 (0)	35 (10)	21 (23)	12 (33)	0 (43)
	0	20	40	60	80

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